Introgression between *Elymus caninus* and *E. fibrosus* as Revealed by Morphology and One-Dimensional SDS-Electrophoresis

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Abstract: Artificial sexual hybrids between *Elymus caninus* and *E. fibrosus* were studied in the F_2–F_5 generations. Each generation of hybrids were characterized by their SDS-electrophoresis patterns of the seed storage proteins (the prolamine-gluteline complex). All plants were grown under open pollination. The possibility of genetic introgression between *E. caninus* and *E. fibrosus* was shown in hybrids between two accessions from the Altai (BEL-9308 × BSK-9302). Morphologically, the F_1–F_5 hybrids were similar to either one of parental species or to *E. caninus* var. *muticus*. The polypeptide spectra of endosperm proteins in the SDS-PAGE were described for the different accessions of the parental species and their hybrid progenies. The results indicate that two species studied belong to a common introgression gene pool (IGP), and that stabilization of their seed fertility seems to occur in the F_4 generation. Our conclusions are that inter-specific introgression is possible after backcrossing and that a similar mechanism of introgression in nature could result in recurrent formation of new genotypes.

Keywords: introgression; hybridization; SDS-electrophoresis; *Elymus caninus*; *Elymus fibrosus*

Elymus caninus L. and *E. fibrosus* (Schrenk) Tzvel. are perennial, self-fertilizing allotetraploid species with identical genomic constitution of StH haplomes (Dewey 1968, 1984; Wang *et al*. 1994). Their areas are overlapping in Eurasia over vast territory from Norway to East Siberia and Central Asia (Tzvelev 1976; Hultén & Fries 1986; Peshkova 2001).

According to the literature two taxa represent morphologically well distinguishable species. The main morphological distinctions of *E. fibrosus* from *E. caninus* are narrower leaves, absence of lemma awns, and fewer flowers in a spikelet. In addition, the habitats of these two species tend to differ: *E. fibrosus* grows on mainly little-moistened and well drained soils in open meadow slopes and valley meadows whereas *E. caninus* grows in more moist, shaded sites.

It was earlier shown that Siberian populations of *Elymus caninus* had a significant variation in polypeptide spectra of endosperm proteins as compared with *E. fibrosus* (Kostina *et al.* 1998; Gerus & Agafonov 2003). The allozyme diversity in a single population of *E. caninus* from Denmark was much higher than the diversity values detected in *E. fibrosus* at a larger geographical scale (Sun *et al*. 2001).

Sometimes in herbaria and collections of living plants of Botanical Institutions there are natural accessions which may be identified as intermediate forms of hybrid origin. Some of these are self-fertile and form the basis for stable micropopulations.

The term "introgressive hybridization" was first used by Anderson and Hubricht (1938). Some consider introgressive hybridization to be widespread and important in the evolution of flowering plants (e.g., Anamthawat-Jónsson K. (2001): Molecular cytogenetics of introgressive hybridization in plants. Methods in Cell Science, **23**: 139–148. 2001); others think that hybrids make negligible contributions

and introgression is simply evolutionary noise (Briggs & Walters 1997). We cannot agree with the latter. Suffice it to recall that most of taxa of the tribe *Triticeae* are of hybrid and introgressive origin.

The aim of our work was to study the possibility of interspecific introgression between *E. caninus* and *E. fibrosus* by morphological and electrophoretic analyses of F_2–F_5 generations from populations in which all stages of sexual reproduction was controlled.

MATERIALS AND METHODS

Plant material. Interspecific hybrids between biotypes of *Elymus caninus* (Altaian accession BEL-9308) and *E. fibrosus* (accession of Finnish origin H 10339 and Altaian BSK-9302) were developed in the greenhouse. Plant crossing procedures were carried out following Lu and Bothmer (1990) with modifications.

Plants of *Elymus caninus* var. *muticus* (Holmb.) Karlsson grown in the experimental plot from the seeds of accession H 3928 (received from Dr. B. Salomon, Alnarp, Sweden) were identified as spontaneous pollen donor. The analysis of reproductive properties and morphological characters of hybrid individuals in generations was performed on the plants grown outdoors and in the greenhouse.

Electrophoresis. Preparation of endosperm protein extracts and SDS-electrophoresis were carried out according to Laemmli (1970) with modifications (Kostina *et al*. 1998). The data matrix was also created as explained in the latest.

RESULTS AND DISCUSSION

Hybrids F_1 between *Elymus caninus* from Altai and *E. fibrosus* from Finland in direct and reciprocal variants of hybridization (H 10339 × BEL-9308 and BEL-9308 × H 10339) were completely sterile. Data were checked during two growing periods in outdoor conditions. The hybrids were well developed and formed more than 120 spikes in all. They were morphologically intermediate between the parental plants in most respects: spikes were laxer, awns of lemmas were about 8 mm, leaves were narrower than those of *E. caninus*. In plant height, the hybrids exceeded both parental biotypes.

All further data are for the hybrids between the Altaian biotypes of *E. caninus* and *E. fibrosus* (BEL-9308 × BSK-9302). An overall scheme of hybrids in generations is shown in Figure 1.

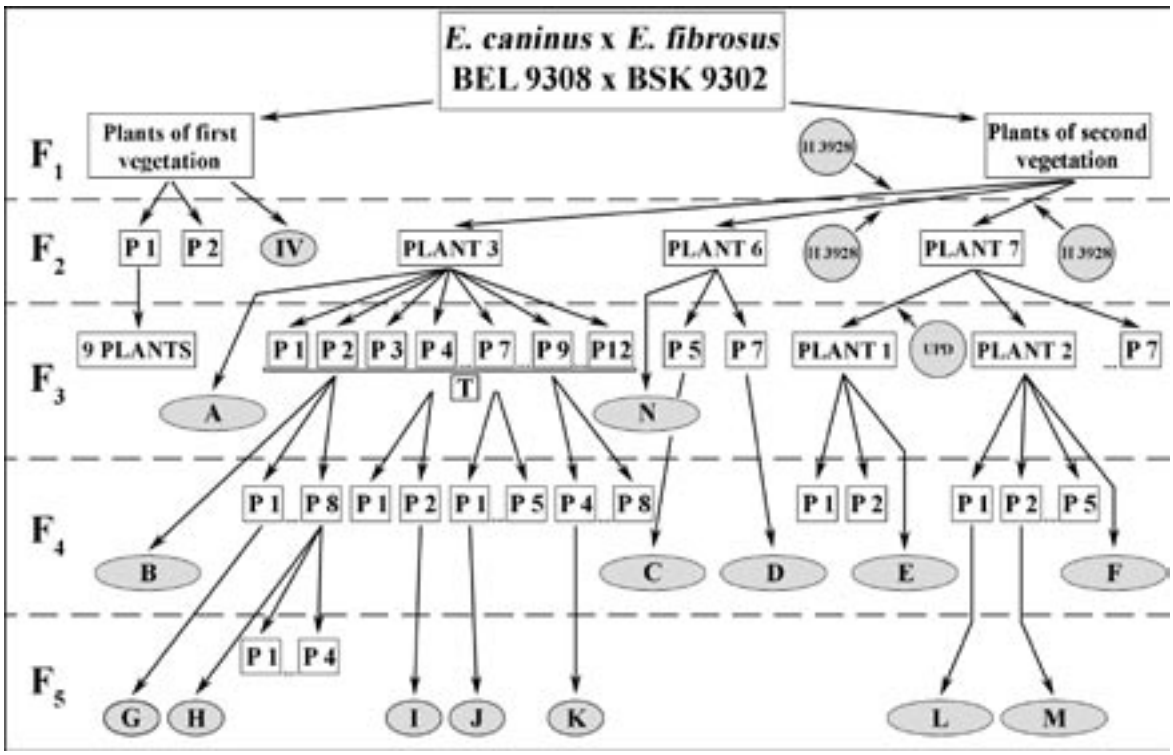

Figure 1. The scheme of electrophoretic and morphological analyses of the interspecific hybrid BEL-9308 × BSK-9302 (UPD – unidentified pollen donor)

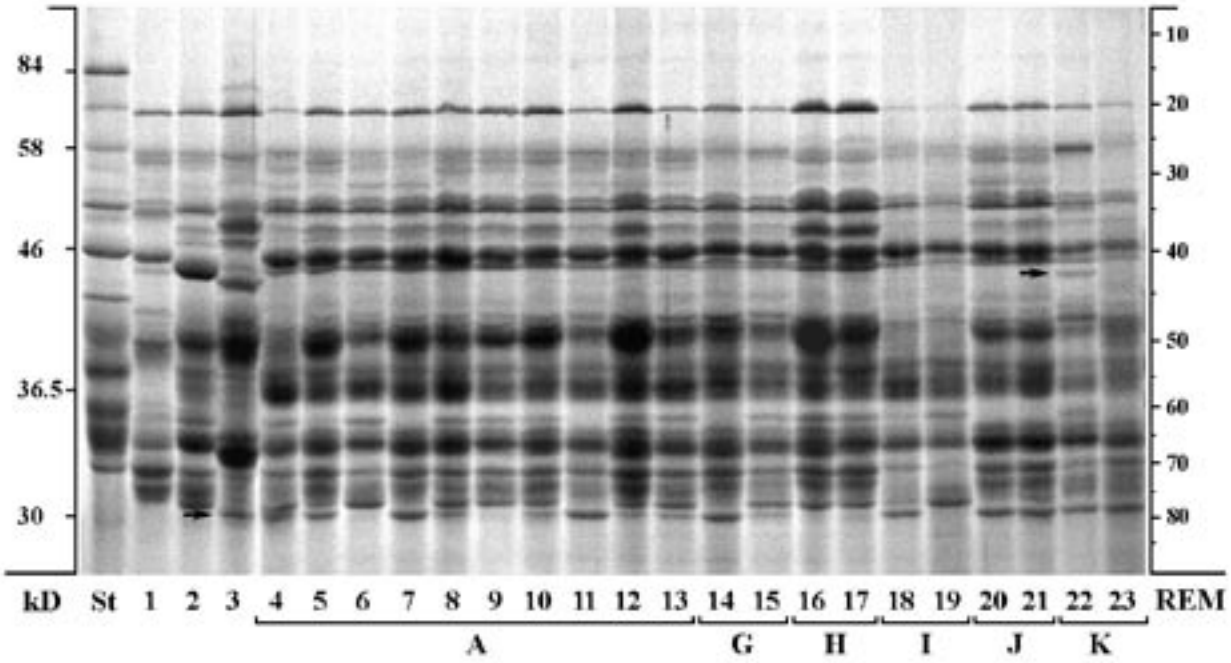

Figure 2. SDS-PAGE of endosperm proteins of the interspecific hybrid *E. caninus* × *E. fibrosus* (BEL-9308 × BSK-9302) as compared with parental biotypes BEL-9308 (2) and BSK-9302 (3) and biotype *E. caninus* var. *muticus* H 3928 (1). The polypeptide spectra of individual seeds in generations in the variant +Me

A – F_3 from plant F_2 – (3); G – F_5 from plants F_4 – (1), F_3 – (2), F_2 – (3); H – F_5 from plants F_4 – (8), F_3 – (2), F_2 – (3); I – F_5 from plants F_4 – (2), F_3 – (4), F_2 – (3); J – F_5 from plants F_4 – (1), F_3 – (7), F_2 – (3); K – F_5 from plants F_4 – (4), F_3 – (9), F_2 – (3)

Morphological analysis. Two F_1 hybrid individuals formed 3 plump seeds during the first field season. One of two F_2 plants grown was absolutely sterile, the other formed 12 seeds. None of the F_3 plants (9 individuals) produced seed. They varied in their morphological characteristics, e.g., the leaf blades varied from densely pubescence to glabrous, the lemmas from glabrous to long pubescent, and the lemma awns from 4 to 15 mm in length. The anthers of all 9 plants were indehiscent and no seeds were formed.

The plants of subsequent generations grown from seeds of F_1 hybrids produced in the second field season were tested according to the scheme shown in Figure 1. Seeds from the parental biotypes and those from plants in later generations (letter symbols) were compared electrophoretically. These comparisons also included seeds of the accession *E. caninus* var. *muticus* H3928 which had been identified as a identified spontaneous pollen donor.

In all plants "T" lemmas were glabrous, structure of glumes did not differ from those of parental forms of *E. caninus* and E. *fibrosus* and spike density differed insignificantly depending on position on a plant. In this case a diagnostically important character "pubescence of leaf blades" manifested in "T" in all intermediate expressions from glabrous to densely hairy reflects spontaneous hybridization with pollen donor H 3928. Most of the F_4 plants were matched on of the parental species or *E. caninus* var. *muticus*, H 3928, in the characters used for identification but they exhibited considerable variation in others characters.

Electrophoretic analysis

Three descending generation lines from plants 3, 6 and 7 were studied. Electrophoretic analysis showed that in progeny of these plants, polypeptide components of biotype of *E. caninus* var. *muticus* H 3928 were found. It means that all three plants originated after spontaneous pollination of the hybrid F_1 by pollen of the donor H 3928. Such additional crossings inject a balanced chromosomal set into a zygote which promotes normalization

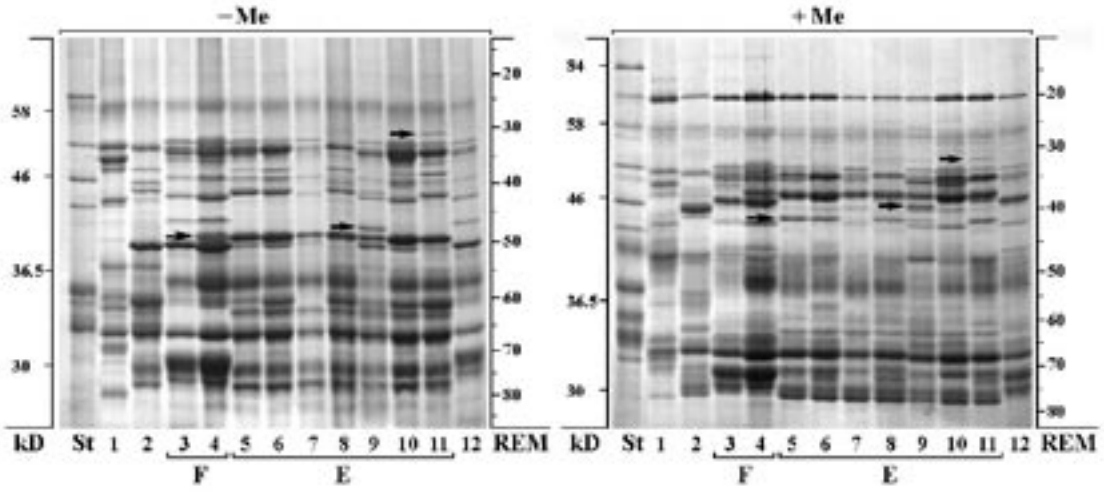

Figure 3. SDS-PAGE of endosperm proteins of the interspecific hybrid *E. caninus* × *E. fibrosus* (BEL-9308 × BSK-9302) as compared with parental biotypes BSK-9302 (1) and BEL-9308 (2) and biotype *E caninus* var. *muticus* H 3928 (12). The polypeptide spectra of individual seeds F_4 in the variants –Me (left) and +Me (right)

F – F_4 from plant F_3 – (2), F_2 – (7); E – F_4 from plant F_3 – (1), F_2 – (7)
Arrows mark polypeptides have not been revealed in parental biotypes

of meiosis and stabilization of seed fertility of plants F_3. It is probable that the lack of spontaneous pollination of the F_1 plants during the first field season was the reason for the absence of stabilization of seed fertility of this descending line .

The polypeptide composition of spectra in seeds of generations F_3 ("A") and F_5 ("G", "H", "I", "J", "K") of the same F_2 plant (Plant 3) was most similar to that of *E. caninus* BEL-9308 and spontaneous donor H 3928 (Figure 2). Marker polypeptide REM 78 of *E. fibrosus* BSK-9302 was found in "A" (except for 6 and 9), in "G" (14), "I" (18), "J", and "K". In the grain of "K" (22) a component coinciding with BSK-9302 of REM 42–43 was found (marked by an arrows).

Electrophoretic comparison of samples "F" and "E" of F_4 from the F_2 plant (Plant 7) (Figure 3) revealed recombination of polypeptide components of the parental biotype BSK-9302 and spontaneous pollen donor H 3928 in two sets of samples. Components not belonging to any of the parental forms were found in grain 4 from "F" and in all seeds from "E" in the variant –Me (Figure 3, left) of REM 49 (marked by an arrow). Their origin is most likely from an unidentified spontaneous pollen donor (USD). A component of REM 31 belonging to none of the parental forms was also found in grain 11 from "E".

After treatment of the extracts by 2-mercaptoethanol (variant +Me, Figure 3, right), the components of REM 49 (Figure 3, left) changed electrophoretic mobility to REM 42 (marked by an arrow).

Electrophoretic analysis of random seed samples "B", "N", "C", "D" and one hybrid grain F_2 from the plant F_1 – 2 (IV) from the plant of the first field season showed that the polypeptide components of BEL-9308 and spontaneous pollinator H 3928 were present in these samples of seeds. Only two components of *Elymus fibrosus* BSK-9302 showed themselves in the spectrum of the F_2 grains (IV). The polypeptide components coinciding with BSK-9302 was found in only one grain from "N".

Thus, the electrophoretic analysis of endosperm proteins of hybrid BEL-9308 × BSK-9302 and subsequent generations has shown that polypeptides of only one parent, *Elymus caninus* BEL-9308, are present in most of the seed samples studied. A few polypeptides unique to the biotype of *E. fibrosus* BSK-9302 were found in samples "A", "G", "H", "I", "J", and "K". It seems that most of the genetic systems controlling protein synthesis in BSK-9302 were eliminated by natural selection. However, analysis of the polypeptide components in succeeding generations showed an increase in randomness. If to obtain F_2 plants, single seeds were sown then, as fertility increased, there is an increased degree of randomness in selection of seeds for formation of a subsequent generation. Thus, high heterogeneity of seed samples "E" was most likely a consequence of spontaneous cross-pollination of the maternal

plant (1) of the F_3 generation as the anthers of this plant were mostly indehiscent. Two daughter individuals grown from sister seeds of samples "E" were completely sterile. In addition, the polypeptide composition of patterns in hybrid seeds is not always a consequence of simple combination of proteins of initial parental biotypes. Along with additive heredity of polypeptide chains in complex genotypes, intergenomic interaction manifested as suppression and derepression of certain genes may take place (Sozinov 1985).

The most significant conclusion of this study is that the two species studied, *Elymus caninus* and *E. fibrosus*, belong to a common introgression gene pool (IGP) (Agafonov & Salomon 2002), and that stabilization of seeds fertility seems to occur in the generation F_4. We conclude that inter-species introgression is possible after backcrossing and that a similar mechanism of introgression in nature could result in recurrent formation of new genotypes. Furthermore, the possibility of genetic introgression allows use of the gene pools of the two species for breeding of new forage cultivars.

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References

Agafonov A.V., Salomon B. (2002): Genepools among SH genome *Elymus* species in boreal Eurasia. In: Hernández P. *et al*. (eds): Triticeae IV. Consejeria de Agricultura y Pesca, Sevilla, Spain: 37–41.

Anamthawat-Jónsson K. (2001): Molecular cytogenetics of introgressive hybridization in plants. Methods in Cell Science, **23**: 139–148.

Anderson E., Hubricht L. (1938): Hybridization in Tradescantia. III. The evidence for introgressive hybridization. American Journal of Botany, **25**: 396–402.

Briggs D., Walters S.M. (1997): Plant Variation and Evolution. Cambridge University Press, Cambridge.

Dewey D.R. (1968): Synthetic *Agropyron-Elymus* hybrids. III. *Elymus canadensis* × *Agropyron caninum*, *A. trachycaulum* and *A. striatum*. American Journal of Botany, **55**: 1133–1139.

Dewey D.R. (1984): The genomic system of classification as a guide to intergeneric hybridization with the perennial *Triticeae*. In: Gustafson J.P. (ed.): Gene Manipulation in Plant Improvement. Plenum Publ. Corp., New York, U.S.A., 209–279.

Gerus D.E., Agafonov A.V. (2003): Variation of *Elymus fibrosus* (Triticeae: Poaceae) in endosperm protein polypeptide spectra detected by SDS-electrophoresis in comparison with the data in *E. caninus*. In: Problems of Botany of Southern Siberia and Mongolia. In: Proc. 2nd Int. Conf., Barnaul, Russia, 25–28.

Hultén E., Fries M. (1986): Atlas of North European Vascular Plants North of the Tropic of Cancer. Koelts Sci. Books, Königstein.

Kostina E.V., Agafonov A.V., Salomon B. (1998): Electrophoretic properties and variability of endosperm proteins of *Elymus caninus* (L.) L. In: Jaradat A.A. (ed.): Triticeae III. Science Publishers, Enfield, New Hampshire, U.S.A., 265–272.

Laemmli U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, **227**: 680–685.

Lu B.-R., von Bothmer R. (1990): Intergeneric hybridization between *Hordeum* and Asiatic *Elymus*. Hereditas, **112**: 109–116.

Peschkova G.A. (2001): *Elymus*. In: Malyschev L.I., Peschkova G.A. (eds): Flora of Siberia. *Poaceae* (*Gramineae*). Science Publishers, Inc., Enfield, New Hampshire, U.S.A., **2**, 11–25.

Sozinov A.A. (1985): Polymorphism of Protein and its Significance for Genetics and Breeding. Nauka, Moscow, 272.

Sun G.-L., Diaz O., Salomon B., von Bothmer R. (2001): Genetic diversity and structure in a natural *Elymus caninus* population from Denmark based on microsatellite and isozyme analyses. Plant Systematics and Evolution, **227**: 235–244.

Tzvelev N.N. (1976): Zlaki SSSR. Nauka, Leningrad.

Wang R.R.C., von Bothmer R., Dvorak J., Fedak G., Linde-Laursen I., Muramatsu M. (1994): Genome symbols in the *Triticeae* (*Poaceae*). In: Wang R.R-C, Jensen K.B., Jaussi C. (eds): Proc. 2nd Int. Triticeae Symp., Logan, Utah, U.S.A.: 29–34.